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Influence of route of administration on progesterone metabolism

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Abstract

While inducing similar progesterone levels, the ratio of 5α- and 5β-pregnanolone/progesterone plasma concentrations has been dramatically increased after oral as opposed to vaginal administration, in a cross-over study. The known psychotropic effects of 5α- and 5β-pregnanolone lead to different indications and precautions for oral and vaginal administration of progesterone.

Keywords: Vaginal administration; Oral administration; Progestones; 5α-pregnanolone; 5β-pregnanolone

1. Introduction

Progesterone and related synthetic compounds called progestins are currently used to control the endometrium, in order to either improve or suppress fertility, to achieve optimum secretory transformation or to prevent hyperplasia during endogenous or exogenous estrogen stimulation. It is now well recognized that each steroid may have different side effects, and some specific characteristics are attributed to natural progesterone, i.e. no suspected harmful effect on atherogenesis, nor on vasomotion [1], specific modulation of androgens and aldosterone activities [2,3], and no suspected teratogenic effects [4].

Progesterone can also be successfully used to induce either amenorrhoea or regular bleeding, subatrophy or full secretory changes, by modifying the dose and duration of treatment and the route of administration [5,6]. Specific binding sites for progesterone have been identified, not only in the uterus, but also in the brain, with specific accumulation in the cerebral cortex, hippocampus and hypothalamus. Moreover, progesterone can be synthesized and metabolized in glial cells and some of its metabolites may react with the $GABA_A$ receptor complex, potentially eliciting hypnotic, anxiolytic or anti-epileptic effects [7] and also producing changes in prolactin and GnRH biosynthesis.

The 5α- or 5β-pregnane-3α-ol-20-one metabolites seem specifically effective in enhancing $GABA_A$ activity [8,9]. While most synthetic pro-

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gestins seem to have somewhat negative effects on mood [10], progesterone has been described as having no effects [11], positive — i.e. mainly anxiolytic — influence [12], or as a drug inducing sedative and hynoptic effects [13]. This latter hypnotic effect is not observed with vaginal administration, whatever the dose, but has been reported with oral administration of relatively large doses of progesterone [13–15]. Such clinical observations suggest possible differences in progesterone metabolism according to the route of administration, and specifically different levels in pregnanolone 5α- or 5β- metabolites [13,15]. Therefore, the same natural steroid, progesterone, may have different effects on the central nervous system, according to whether there is oral or vaginal administration, and different routes may be preferred for different indications or to modify side effects.

In a previous report, progesterone plasma concentrations have been compared in a randomized cross-over study after oral and vaginal administration of progesterone [15]. The present data concerns plasma levels of several progesterone metabolites obtained from the same study.

2. Materials and methods

2.1. Subjects

Nine healthy female volunteers were included in the study. They were premenopausal with regular menstrual cycles, having no history of renal, hepatic or gastro-intestinal disease and no evidence of vaginal ulcers, inflammation or infection. They were within 15% of ideal body weight and using adequate contraceptive methods that did not involve the use of hormonal compounds. All subjects gave written informed consent and the study was approved by the Austin Hospital Human Ethics Committee.

2.2. Treatment

The study was a cross-over design using progesterone in either an oral or a vaginal formulation. The oral formulation was in the form of soft gelatin capsules containing 100 mg of micronized progesterone in oil (Utrogestan, Besins-Iscovesco, Paris, France). The vaginal formulation was a waxy suppository containing 400 mg progesterone in a base of semi-synthetic glycerides produced from hydrogenated vegetable oil by interesterification (Cyclogest, Cox Pharmaceuticals, Barnstaple, United Kingdom). Random allocation of subjects to either a single oral (2 × 100 mg capsules) or vaginal (1 × 400 mg suppository) dosing, followed by the alternate dosing form, was used. The single administration was done between day 4 to 6 of the menstrual cycle to ensure low baseline values of endogenous progesterone. Only one formulation was administered during one menstrual cycle and the study was conducted over two cycles in each subject.

Volunteers were instructed to refrain from sexual intercourse the day before and until 4 days after vaginal administration. They were required to abstain from caffeine beverages 24 h before treatment. On the first day of the study volunteers were provided with a standardized breakfast before 08:00, followed by progesterone administration at 08:30. Subjects remained in a semi-reclined position for 4 h after vaginal administration. Blood samples for progesterone metabolite analysis were collected before the initial progesterone dose and at 1, 2, 3, 4, 6 and 8 h after the dose from an in-dwelling heparinized catheter, inserted into an arm vein. Blood was collected in lithium heparin tubes, centrifuged immediately and stored frozen at −20°C until analysis.

2.3. Steroid analysis

The determination of 5α- and 5β-pregnanolone was performed by gas chromatography-mass spectrometry with stable isotope dilution [16]. Predetermined doses of deuterium labelled analogues were added to plasma samples. Extracts were purified by liquid chromatography on Sephadex LH-20. Derivatized and selected ion monitoring was performed at nominal masses m/z 496 and 500, corresponding to the characteristic ions of the heptaflurobutyrates of the native and labelled pregnanolones. Intra-assay variability was between 3 and 5% for concentrations of 1 ng/ml or more and between 7 and 10% for concentrations below 0.5 ng/ml. Unconjugated steroids were determined by specific radioimmunoassays after chromatographic separation of plasma extracts,

Table 1
Oral progesterone

	Cmax (ng/ml)	Tmax (h)	AUC (0–8 h)	Mean plasma (0–8 h) (ng/ml)
Progesterone	19.00 ± 11.48	3.44 ± 0.96	46.16 ± 22.20	5.77 ± 2.78
5α-Pregnanolone	33.66 ± 17.77	3.25 ± 0.83	92.92 ± 38.80	12.87 ± 6.09
5β-Pregnanolone	19.99 ± 10.14	3.00 ± 1.05	51.44 ± 31.42	6.91 ± 4.20
5α-DHP	9.32 ± 5.44	3.11 ± 1.91	23.78 ± 11.48	2.97 ± 1.43
11-DOC	1.05 ± 0.55	3.67 ± 1.05	3.54 ± 1.17	0.44 ± 0.15

performed on celite columns for P, DOC (11-deoxycorticosterone) and 5α-dihydroprogesterone (DHP).

3. Results

Mean Cmax, Tmax, AUC, and mean plasma values measured during the 8 h survey are indicated in Table 1 and Table 2. The area under the curve was calculated using the trapezoidal rule.

3.1. Progesterone

Within the 8 h of blood sampling, the area under the curve (AUC), maximum plasma concentrations (Cmax) and mean plasma values were similar after oral and vaginal administration. Time to maximum concentration (Tmax) tended to be shorter after oral (3.4 ± 0.96 h) than after vaginal administration (4.89 ± 2.28), but the difference was not significant (Fig. 1).

3.2. 5α-Pregnanolone

After oral administration, 5α-pregnanolone had similar Tmax to progesterone (3.25 ± 0.83 h) but significantly higher AUC, Cmax (33.66 ± 17.7 ng/ml) and mean plasma values (12.87 ± 6.09 ng/ml).

After vaginal administration, 5α-pregnanolone was significantly increased at 6 and 8 h only in comparison with baseline (Tmax = 7.33 ± 1.33 h). Plasma values were consistently and significantly lower after vaginal administration (Figs. 2 and 3).

3.3. 5β-Pregnanolone

After oral administration, 5β-pregnanolone had similar Tmax (3 ± 1.05 h) to progesterone and 5α-pregnanolone. AUC, Cmax (19.99 ± 10.14 ng/ml) and mean plasma values (6.91 ± 4.20 ng/ml) were similar to progesterone values but significantly and consistently lower than 5α-pregnanolone values from 2 to 8 h. After vaginal administration, no significant increase in 5β-pregnanolone plasma values was observed.

3.4. 5α-DHP

After oral administration, 5α-DHP had similar Tmax (3.11 ± 1.91 h) but significantly lower

Table 2
Vaginal progesterone

	Cmax (ng/ml)	Tmax (h)	AUC (0–8 h)	Mean plasma (0–8 h) (ng/ml)
Progesterone	16.30 ± 17.15	4.89 ± 2.28	54.72 ± 32.08	7.46 ± 5.61
5α-Pregnanolone	1.16 ± 0.71	7.33 ± 1.33	5.11 ± 2.24	0.64 ± 0.28
5β-Pregnanolone	0.26 ± 0.10	4.22 ± 3.33	1.37 ± 0.65	0.17 ± 0.08
5α-DHP	0.76 ± 0.75	6.56 ± 2.27	3.44 ± 3.29	0.44 ± 0.41
11-DOC	0.30 ± 0.22	3.44 ± 2.63	1.44 ± 0.79	0.19 ± 0.14

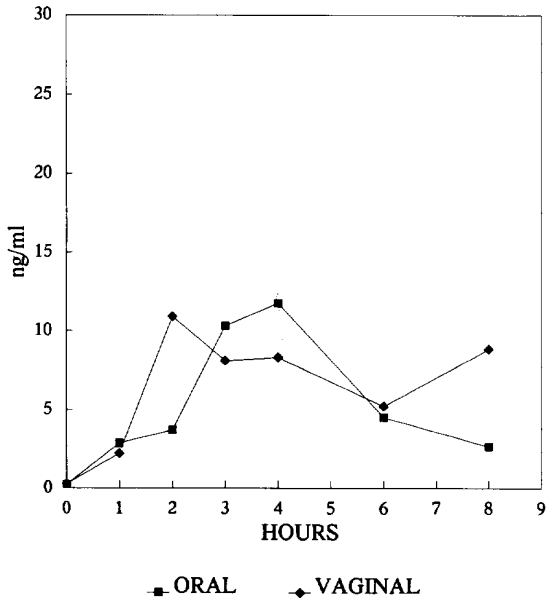

Fig. 1. Progesterone plasma levels (ng/ml).

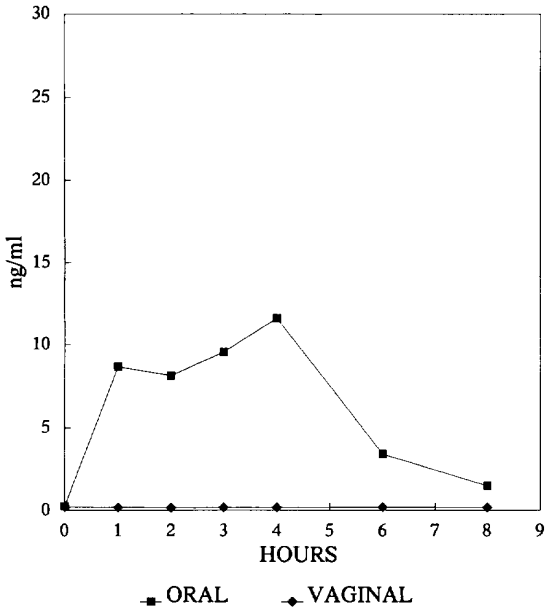

Fig. 3. 5β-Pregnanolone plasma levels (ng/ml).

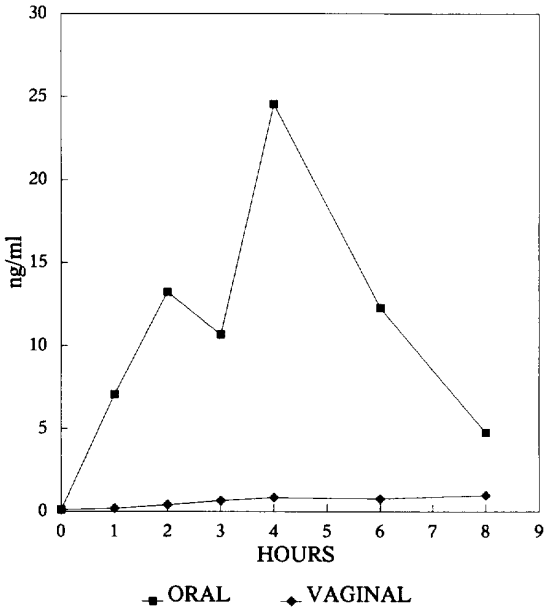

Fig. 2. 5α-Pregnanolone plasma levels (ng/ml).

AUC, Cmax (9.32 ± 5.44) and mean plasma values than progesterone, 5α- and 5β-pregnanolone. After vaginal administration, a slight significant increase was observed between 2 and 8 h in comparison with baseline. Mean 5α-DHP plasma values were significantly lower after vaginal administration.

3.5. DOC

After oral administration, DOC levels were significantly increased between 3 and 8 h in comparison with baseline. DOC had similar Tmax (3.67 ± 1.05 h) than progesterone but significantly lower AUC, Cmax (1.05 ± 0.55 ng/ml) and mean plasma values (0.44 ± 0.15 ng/ml). After vaginal administration, no significant increase in DOC plasma values was observed.

4. Discussion

Besides classical effects on the uterus, antiepileptic, anxiolytic, hypnotic and anesthetic properties have been attributed to progesterone, ac-

cording to numerous animal and human studies [17–19]. Most of these studies suggest that the reported effects on the central nervous system are more likely related to some pregnane metabolites than to progesterone itself. In particular, the 5α- and 5β-pregnane-3α-ol-20-one metabolites have been demonstrated to induce almost instant anesthetic effects, faster and stronger than those induced by progesterone [17,20,21]. Intravenous administration of 5β-pregnanolone, as a bolus dose ranging from 0.4 to 0.6 mg/kg induces sleep and anaesthetic effects within a few seconds in men [22]. These metabolites do not act through the progesterone nuclear receptor but through the $GABA_A$ membrane receptor and are reported to be 700–1000 times more potent than most active barbiturates in stimulating the binding of benzodiazepines or inhibiting the binding of convulsant drugs [7]. Therefore, some effects of progesterone on the central nervous system may be quantitatively or qualitatively different, according not only to the dose but also to the route of administration which elicit different metabolization processes.

Orally-administered progesterone undergoes several successive metabolization steps in the gut, intestinal wall and liver [23]. The first step is the contact with intestine bacteria which has 5β-reductase activity, then with the intestinal wall which has 5α-reductase activity and also initiates conjugation of steroids with glucuronic acid. The second step is the contact with liver enzymes after circulation in the portal vascular systems. Liver cells in women express mainly 5β-reductase, 3α- and 20α-hydroxylase activities and are also able to conjugate steroids with glucuronic acid. Only a fraction of the native steroid administered eludes the different enzyme activities and circulates in the plasma as remaining progesterone, while most of the steroids circulate as inactive 5β-pregnane-3α-ol-20α-diol-glucuronide. The native fraction seems negligible when progesterone is slowly absorbed from crystalin formulation by the intestinal tract, but it is significantly increased when the absorption process is sped up by micronization of the steroid [24]. Both high levels of circulating progesterone [15,25] and high levels of pregnanolone metabolites have previously been described after oral administration of micronized progesterone [13,16,26]. By contrast, normal vaginal bacteria and mucosa seem devoid of 5α- and 5β-reductases and 3α- and 20α- hydroxylases and progesterone are absorbed without significant metabolic changes.

In the present cross-over study, while progesterone has been administered in doses capable of inducing similar plasma levels of the native steroid during the 8 h following either oral or vaginal administration, circulating metabolites were significantly different. After oral administration, 5α-pregnanolone levels were even higher than those of progesterone and 5β-pregnanolone levels were similar to those of progesterone. By contrast, after vaginal administration, only a small increase in 5α-pregnanolone, ~ 10% of the oral increase, was observed and 5β-pregnanolone levels were not significantly affected. These results strongly support the concept that progesterone activities on the central nervous system can be modulated by the route of administration. The rate of side effects, such as drowsiness, clearly differs according to the route of administration [15], even if these side effects seems to occur similarly following progesterone or placebo when administered at bedtime [27].

More interesting, different benefits and indications may be expected from the two routes of administration. For example, specific benefit may be expected from oral administration on mood and sleep disturbances in postmenopausal women [14] or in women suffering from premenstrual symptoms of anxiety [12], while vaginal administration may induce more predictable endometrial secretory changes without detectable influence on the central nervous system [5,11]. Assuming that pregnanolone metabolites are physiological, anxiolytic and hypnotic steroids, some of the psychological side effects described with synthetic progestins [10], which are not pro-drugs for these metabolites, may also be explained. The slight but significant increase in DOC plasma levels observed after oral administration in comparison with vaginal administration has no detectable influence since anti-mineralocorticoid effects of progesterone remain dominant. Oral administration of micronized progesterone has been shown to antagonize mineralocorticoid activity of

9α-fluorohydrocortisone [3] and to slightly but significantly decrease blood pressure [28].

In conclusion, oral administration of micronized progesterone not only increases progesterone plasma values but, in addition, 5α- and 5β-pregnanolone levels, which are known to act directly on $GABA_A$ receptors of the central nervous system. These specific metabolites are likely to explain not only some previously observed side effects which are easy to prevent, but also may offer additional therapeutic benefits in some specific indications like mood and sleep disturbances associated with menopause or premenstruum. By contrast, vaginal administration, which is known to be highly effective in inducing secretory endometrium, elicits only minor changes in plasma levels of 'psychotropic' metabolites.

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